AMENDMENTS TO THE TITLE:

Please delete the Title on the first and final (page 58) pages of the specification and replace it with the following Title:

GRAFT POLYMERS, THEIR PREPARATION AND USE IN CAPILLARY ELECTROPHORESIS

AMENDMENTS TO THE SPECIFICATION:

Please amend the specification as follows:

Page 1, paragraph beginning on line 8 and ending on line 13:

-- The invention relates generally to graft polymers ~~copolymers~~, their preparation, and electrophoresis separation compositions comprising the same; also to ultra-high molecular weight poly(*N,N*-dimethylacrylamide) polymers, their preparation, and electrophoresis separation compositions comprising the same; and more particularly to supports, such as capillaries, containing these polymers and methods for separating biomolecules, especially polynucleotides, using capillary electrophoresis. --

Paragraph beginning on page 4, line 31 and ending on page 5, line 7:

-- "The invention relates generally to graft polymers ~~copolymers~~, their preparation, and compositions comprising the same; also to ultra-high molecular weight poly(*N,N*-dimethylacrylamide) polymers, their preparation, and compositions comprising the same; and more particularly to supports, such as capillaries, containing these polymers and methods for separating biomolecules, especially polynucleotides, using capillary electrophoresis. The graft polymers ~~copolymers~~ are surprisingly and unexpectedly effective in, e.g., electrophoresis separation media and for use in CE as dynamic coating polymers that effectively suppress electroosmosis or electroosmotic flow ("EOF"), which refers to capillary fluid flow induced by an electrical field. An exemplary graft polymer ~~copolymer~~ of the invention can be described as a poly(DMA) ("PDMA") backbone polymer bearing polyacrylamide ("PAAm") side-chains or pendant chains. --

Page 5, paragraph beginning on line 7 and ending on line 16:

-- As used herein, a "copolymer" includes a polymer comprising at least two different monomeric subunits. Thus, a polymeric chain made up of three different monomers (also known as a terpolymer) is included within the term "copolymer," as are polymer chains containing more than three different monomeric units. Copolymers may be formed in many ways known to those of ordinary skill in the art, for example: by polymerizing two different monomers; by block copolymerization; by graft polymerization ~~copolymerization~~, e.g., where an existing copolymer ~~polymer~~ chain is further reacted with a different monomer; and by a post-polymerization reaction, e.g., where a polymer with ester side groups is partially

hydrolyzed. As used herein, the term "polymer" includes a homopolymer and a copolymer. --

Page 5, paragraph beginning on line 17 and ending on line 26:

-- It is conventional in the polymer art that graft polymers ~~copolymers~~ are commonly named as "poly(M_1-g-M_2)," where M_1 refers to the monomer or monomers making up the backbone polymer or backbone copolymer, i.e., poly(M_1); and M_2 (following the "g" for graft) refers to the monomer or monomers making up the grafted polymer or grafted copolymer, i.e., "poly(M_2)," sometimes also referred to herein as the pendant, pendant polymer, pendant chain or side-chain polymer. It is to be understood that poly(M_1) and poly(M_2) can be the same or different homopolymer or copolymer. See G. Odian, *Principles of Polymerization*, McGraw-Hill Book Co., New York, 1970, pp. 366, 633 and U.S. Patent No. 6,319,976 B1 to DeNicola, Jr. et al. for a further explanation and examples of this nomenclature. --

Paragraph beginning on page 5, line 27 and ending on page 6, line 2:

-- For example, poly(*N,N*-dimethylacrylamide-g-acrylamide) denotes a graft polymer ~~copolymer~~ where the backbone polymer is poly(*N,N*-dimethylacrylamide) and the pendant polymer is poly(acrylamide), poly((*N,N*-dimethylacrylamide-co-*N,N*-diethyl-methacrylamide)-g-acrylamide) denotes a graft polymer ~~copolymer~~ where the backbone polymer is a copolymer of *N,N*-dimethylacrylamide and *N,N*-diethyl-methacrylamide and the pendant polymer is poly(acrylamide), and poly(*N,N*-dimethylacrylamide-g-(acrylamide-co-*N*-butoxymethyl-methacrylamide-co-*N*-methoxymethyl-acrylamide) denotes a graft polymer ~~copolymer~~ where the backbone polymer is poly(*N,N*-dimethylacrylamide) and the pendant polymer is a copolymer of acrylamide, *N*-butoxymethyl-methacrylamide and *N*-methoxymethyl-acrylamide. --

Page 6, paragraph on line 4:

-- 5.1 Graft Polymer ~~Copolymer~~ Poly(M_1-g-M_2) --

Page 6, paragraph beginning on line 5 and ending on line 6:

-- The first embodiment of the invention relates to graft polymer ~~copolymer~~ poly(M_1-g-M_2) or a salt thereof, where: --

Page 10, paragraph beginning on line 5 and ending on line 6:

-- In an embodiment of the invention, the graft polymer ~~copolymer~~ is poly(M_1-g-M_2) or a salt thereof, where: --

Page 10, paragraph beginning on line 34 and ending on line 35:

-- In another embodiment of the invention, the graft polymer ~~copolymer~~ is poly(M_1-g-M_2) or a salt thereof, where: --

Page 11, paragraph beginning on line 28 and ending on line 29:

-- In another embodiment of the invention, the graft polymer ~~copolymer~~ is poly(M_1-g-M_2) or a salt thereof, where: --

Paragraph beginning on page 13, line 29 and ending on page 14, line 6:

-- In another embodiment, a graft polymer ~~copolymer~~ of the invention is water soluble, water swellable or both, at atmospheric pressure, a concentration of from about 0.01 to about 1 wt.%, and from about 20°C to about 70°C, e.g., at 25°C. For purposes of this invention, water swellable graft polymers ~~copolymers~~ are generally either those that swell in water but appear not to be completely soluble because they have a very slow dissolution rate, e.g., graft polymers ~~copolymers~~ that are substantially uncrosslinked but have an extremely high weight-average molecular weight; or those unable to dissolve completely in water because they have been crosslinked to a certain low degree, for example, by synthesizing the polymer ~~copolymer~~ to comprise certain amounts of crosslinking or branching agents. In one embodiment, a graft polymer ~~copolymer~~ of the invention is substantially uncrosslinked such that it is able to flow into or out of a capillary either with or without the assistance of pressure or vacuum. In another embodiment, a graft polymer ~~copolymer~~ of the invention is substantially uncrosslinked by covalent chemical bonds. --

Page 15, paragraph on line 9:

-- 5.2 Method for Making Graft Polymer ~~Copolymer~~ --

Page 15, paragraph beginning on line 10 and ending on line 20:

-- Many methods of making graft polymers ~~copolymers~~ are known in the art and can be used to prepare the poly(M_1-g-M_2) of the present invention. For example, several such methods are summarized in the following chapter: Costello et al., "Copolymers" in *Kirk-*

Othmer Encyc. of Chem. Technol., 4th Ed., John Wiley & Sons, New York, 1993, Vol. 7, pp. 349-381. These conventional methods include selecting a polymeric backbone with suitable reactive sites, selecting a monomer or monomers, e.g., M_2 of the invention, and then conducting a polymerization, e.g., initiated by free-radical, anionic or cationic means, of that monomer(s) to form a graft polymer ~~copolymer~~. *Id.*, pp. 356-358. Such polymerizations can, of course, be conducted in bulk, solution, suspension, emulsion or microemulsion, and a wide variety of polymerization initiators can be used. *Id.*, p. 356. --

Page 23, paragraph beginning on line 3 and ending on line 16:

-- UHMw PDMA homopolymer (non-graft polymer ~~copolymer~~), i.e., not including poly(M_2), is also effective, e.g., in a CE separation medium for separating biomolecules. Thus, a second embodiment of the invention relates to poly(*N,N*-dimethylacrylamide) where the weight-average molecular weight of the poly(*N,N*-dimethylacrylamide) is at least about 3 MDa. In another embodiment, the sieve polymer is poly(*N,N*-dimethyl-acrylamide) with a weight-average molecular weight of at least about 3 MDa. In another embodiment, the UHMw PDMA has a weight-average molecular weight of from about 3 MDa to about 10 MDa. Additionally, a third embodiment of the invention relates to a method for making ultra-high molecular weight poly(*N,N*-dimethylacrylamide), comprising the step of polymerizing DMA in an inverse emulsion comprising an oil phase, an aqueous phase, a surfactant and an initiator, to provide poly(*N,N*-dimethylacrylamide) with a weight-average molecular weight of at least about 3 MDa. Another embodiment of the invention relates to the poly(*N,N*-dimethylacrylamide) product of this method. --

Page 23, paragraph on line 18:

-- 5.4 Method for Making Graft Polymer ~~Copolymer~~ (Continued) --

Page 23, paragraph beginning on line 19 and ending on line 26:

-- Once a polymeric backbone has been obtained or prepared, a pendant-forming polymerization reaction may be carried out, e.g., by free-radical grafting with monomer or monomers M_2 as discussed above, thereby forming a graft polymer ~~copolymer~~ of the invention. Without being bound by a particular theory, a proposed mechanism for free-radical grafting of an exemplary poly(M_1-g-M_2) of the invention, poly(DMA-g-AAm), is shown in the following scheme. Free-radicals 1, formed, for example, by the thermal or

photolytic decomposition of a free-radical initiator at the start of polymerization, may initiate the polymerization of AAm 2 to form propagating macro-radical 3: --

Paragraph beginning on page 25, line 25 and ending on page 26, line 7:

-- Of course, other ways for initiating polymerization known in the art can also be used to make the poly(M_1-g-M_2) of the invention. For example, exposing a combination of the poly(M_1) and monomer(s) to electron beams, ultraviolet radiation, usually in the presence of a photoinitiator, and high energy ionizing radiation sources, such as γ-radiation from a ^{60}Co or ^{137}Cs source, α-particles, β-particles, fast neutrons and x-rays, can cause the generation of free-radicals and/or ions that, in turn, initiate graft polymerization. Sanchez et al., "Initiators (Free-Radical)," at 454-457; Sheppard et al., "Initiators," in *Kirk-Othmer Encyc. of Chem. Technol.*, 3rd Ed., John Wiley & Sons, New York, 1981, Vol. 13, pp. 367-370. At least the three following radiation grafting methods are conventional: (1) the "pre-irradiation" method, in which the backbone polymer is irradiated before interacting with the monomer(s), (2) the "mutual radiation grafting" method, in which the backbone polymer and the monomer(s) are in contact while irradiation occurs, and (3) the "peroxide" method, in which the backbone polymer is irradiated in the presence of air or oxygen before interacting with the monomer(s). Stannett et al., "Polymerization by High-Energy Radiation" in *Comprehensive Polymer Science*, Pergamon Press, Oxford, 1989, Vol. 4, Eastmond et al., Eds., p. 327-334. Alternatively, it is possible to synthesize graft polymers ~~copolymers~~ using group-transfer polymerization. Costello, p. 359. --

Page 26, paragraph beginning on line 8 and ending on line 18:

-- Another conventional method for preparing graft polymers ~~copolymers~~ that can be used to prepare the poly(M_1-g-M_2) of the invention is the use of telechelic polymers, also known as "macromonomers". Costello, pp. 360-361. As used herein, the term "telechelic polymer" refers to a polymer or oligomer having at least one functional end-group capable of forming bonds with another molecule. For example, a telechelic polymer consisting essentially of vinyl-terminated polymer may be suitably used. The terminal vinyl group of such a telechelic polymer can be polymerized ~~copolymerized~~ with a monomer or monomers, e.g., M_1 of the invention, to form a graft polymer ~~copolymer~~ bearing, as pendant chains, the polymer of the telechelic polymer. Particularly, the telechelic polymer can be vinyl-terminated PAAm which, when polymerized ~~copolymerized~~ with M_1 of the invention, yields poly(M_1-g-acrylamide), i.e., acrylamide as M_2. --

Paragraph beginning on page 26, line 26 and ending on page 27, line 2:

-- Alternatively, the poly(M_1-g-M_2) of the present invention can be prepared from polymeric starting materials, a method that is also conventional in making graft polymers ~~copolymers~~. For example, exposure of a combination of poly(M_1) and poly(M_2) to an ionizing radiation source can lead to the formation of macro-radical intermediates, e.g., by hydrogen abstraction or carbon-carbon bond cleavage. Then, the macro-radical intermediates can couple to form a single, e.g., grafted, polymer ~~copolymer~~ molecule having increased molecular weight relative to the starting polymers. Adler, *Science*, 141:321-323 (1963); McGinniss, "Radiation Curing," in *Kirk-Othmer Encyc. of Chem. Technol.*, 3rd Ed., John Wiley & Sons, New York, 1982, Vol. 19, p. 612. Additionally, reactive processing methods, such as reactive extrusion, can be used to make graft polymers ~~copolymers~~ *in situ* during polymer processing operations performed with a combination of poly(M_1) and poly(M_2). Costello, p. 377. --

Page 42, paragraph beginning on line 2 and ending on line 14:

-- As noted above, the graft polymers ~~copolymers~~ and compositions and separation media containing the same; methods of making the graft polymers ~~copolymers~~ and compositions and separation media containing the same; and methods of using the graft polymers ~~copolymers~~ and compositions and separation media containing the same in CE yield superior CE performance in the analysis and separation of biomolecules. As also noted above, the UHMw PDMA polymers and compositions and separation media containing the same; methods of making the UHMw PDMA polymers and compositions and separation media containing the same; and methods of using the UHMw PDMA polymers and compositions and separation media containing the same in CE yield superior CE performance in the analysis and separation of biomolecules. The following examples further illustrate certain embodiments of the present invention. These examples are provided solely for illustrative purposes and in no way limit the scope of the present invention. --

Page 45, table beginning on line 29 and ending on line 34:

-- **Table 1.** Formulations used for the preparation of poly(DMA-g-AAm)

Graft Polymer ~~Copolymer~~ Designation	AAm (g)	PDMA (g)	Approx. WFR*	Water (g)	2-Propanol (mL)	Ammonium persulfate (g)	Mw (MDa)	Mn (MDa)
GC1	2.0011	2.0024	1	250.0	0.10	0.0085	1.48	0.47
GC2	9.1654	0.9169	10	250.0	0.50	0.0396	2.15	1.02

--

Page 46, paragraph beginning on line 1 and ending on line 4:

-- After 16 hours, the reaction was stopped and the reaction mixture was dialyzed as described above. After lyophilization, 3.7 g of the first graft polymer ~~copolymer~~ ("GC1") was obtained (92% yield) with the following molecular weight as determined by GPC-MALLS: Mw = 1,477,000 Da, Mn = 471,000 Da. --

Page 46, paragraph beginning on line 13 and ending on line 16:

-- As illustrated in Table 1, a higher molecular weight graft polymer ~~copolymer~~ 2 ("GC2") with a different AAm/PDMA ratio was prepared from the same starting materials and using the above procedure except that the amounts of 2-propanol and ammonium persulfate and the feed ratio of AAm to PDMA were varied. --

Page 46, paragraph beginning on line 24 and ending on line 28:

-- Compositions comprising the graft polymers ~~copolymers~~ of the present invention were evaluated for their suitability as capillary electrophoresis separation media in DNA sequencing. In the following examples, each composition and/or separation medium was evaluated in CE by using an ABI 310 Capillary Electrophoresis Gene Analyzer equipped with a 47 cm long by 50 μm inner diameter uncoated fused silica capillary. --

Page 47, paragraph beginning on line 25 and ending on line 34:

-- Compositions of the invention were prepared from poly(DMA-g-AAm), e.g., by the method described above but also using the dialyzed, lyophilized, extracted graft polymer ~~copolymer~~ of this invention. The dialysis procedure was as described above. IC1-3 were each prepared with the same PAAm used in CSM2. IC1 and 2 were each prepared with the graft polymer ~~copolymer~~ GC2 described in Example 6.2 while IC3 was prepared with the acetone-extracted graft polymer ~~copolymer~~ GC1 described in that example. TET-dye labeled

fragment sequencing runs were conducted as described above for the CSM separation media. The composition and CE sequencing performance for each of the compositions of the invention is summarized in Table 3. In Tables 2 and 3, the weight percent values specified are based on the total weight of the composition, i.e., the separation medium. --

Page 48, paragraph beginning on line 13 and ending on line 17:

-- As also illustrated in Table 3, decreasing the amount of PAAm (i.e., the sieve polymer) from 2.02 wt% in IC1 to 1.52 wt% in IC2, with a corresponding increase in the amount of graft polymer ~~copolymer~~ from 0.22 to 0.88 wt%, respectively, also resulted in a higher 50°C crossover value relative to, e.g., CSM1, 637 bp to 532 bp, respectively. At 50°C, IC2 also had run times shorter than or comparable to CSM1 and CSM2. --

Page 48, paragraph beginning on line 18 and ending on line 21:

-- A composition of the invention comprising the lower molecular weight graft polymer ~~copolymer~~ GC1, called IC3, was also effective in CE. IC3 provided an improved crossover value at 50°C of 633 bp, relative to the 532 bp of CSM1 and the 232 bp of CSM2, also with shorter run times. --

Page 48, paragraph beginning on line 24 and ending on line 30:

-- Poly(DMA-g-AAm) graft polymers ~~copolymers~~ of the invention were prepared using free-radical polymerization of acrylamide in an aqueous solution of PDMA, as described in Example 6.2. After dialysis and lyophilization, as also described therein, which removed unreacted acrylamide, initiator and other low molecular weight impurities, only the following polymeric products could have been present in the isolate: (1) poly(DMA-g-AAm); (2) PAAm, which is relatively acetone insoluble; and (3) unreacted PDMA, which is very acetone soluble. --

Page 49, paragraph beginning on line 3 and ending on line 12:

-- The results from DNA sequencing using CE clearly distinguish among separation media containing only PAAm and those containing PDMA and PAAm. A separation medium containing PAAm as the sole polymeric component, i.e., CSM2 in Table 2 above, does not suppress EOF well enough to give a high crossover value. In contrast, the extracted polymer ~~copolymer~~ products IC1-3 of Table 3, when present with PAAm in compositions of the invention used as a separation media, performed markedly better than CSM2, e.g., by

effectively suppressing EOF as indicated by their high crossover values relative to CSM2. This greatly improved CE performance confirms that the PDMA reactant was chemically incorporated into the graft polymers ~~copolymers~~ GC1 and GC2 present in IC1-3. --